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Patent
2694-0119P

In the U.S. Patent and Trademark Office

Applicant:	VOLLENBROICH et al.	Conf.:	9955
Appl. No.:	09/242,343	Group:	1642
Filed:	April 12, 1999	Examiner:	B.Brumback

For: NEW ANTIVIRAL LIPOPEPTIDES, AND A METHOD OF INACTIVATING LIPID-ENVELOPED VIRUSES USING LIPOPEPTIDES

Declaration under 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Georg Pauli, hereby declare the following. I am a co-inventor of the subject matter of the above-identified patent application. I am fully knowledgeable about the field of the invention and am an expert in the fields of virology and viral inactivation. A copy of my *curriculum vitae* is attached hereto. I have also fully reviewed the Office Actions of June 1, 2001 and January 4, 2002 along with the cited references of Itokawa et al., Chem. and Pharmaceutical Bull., 42:604-607 (1994) (hereinafter referred to as "Itokawa et al."), Naruse et al., J. Antibiotics, 43:267-280 (1990) (hereinafter "Naruse et al."), and Horowitz et al., Vox Sanguinis, 54:14-20 (1988) (hereinafter "Horowitz et al."). I have further fully reviewed the reference Weislow et al., J. Natl. Can. Inst. 81:577-586 (1989) (hereinafter referred to as "Weislow et al.").

Considered 7-3-02 GP

In the Office Actions of June 1, 2001 and January 4, 2002, the Examiner has raised the following points about either the nature of the invention or the teachings of one or more of the above listed references which, in my opinion, are scientifically inaccurate.

a) **Level of viral inactivation:** The Examiner asserts that Applicants argue that the claimed method results in full inactivation of virus, but the claims recite a reduction of viral titre of 10^4, i.e. not full inactivation. In my opinion, one skilled in the art would interpret a reduction of viral titre of 10^4 to mean full inactivation of the virus because of the exponential reduction in titre. **The factor of 10^4 is calculated from the titre determined in the sample without the inactivating substance (starting titre) and the titre in the sample after treatment (end titre). When at the end of the treatment no infectious virus can be detected any longer in the assay system, the titre is given as the calculated titre (detection limit) and is given by the term ≤ xy. The reduction factor obtained is therefore limited by the starting titre and the end titre. The lowest reduction factor determined in our experiments was ≥ 10^4 for the inactivation of enveloped viruses with the substance, that means that no virus was detectable after treatment (full inactivation of the virus).**

b) Mechanism of viral inactivation: The Examiner interprets Itokawa et al. as teaching that the XTT formazan assay is used to measure the inactivation of both cell-free HIV-1 and f HIV in chronically infected cells. In addition, the Examiner asserts that the *in vitro* assay of Itokawa et al. that measures viral infectivity is equivalent to measuring virus inactivation.

In my opinion, the Examiner has incorrectly interpreted Itokawa et al. and the use of the XTT formazan assay. Itokawa et al. disclose that surfactins (1) and (2) have moderate anti-HIV activities in the XTT formazan assay for HIV-1 cytopathic effects. See page 607, final paragraph preceding "Experimental" section. As in the case of many journal articles, Itokawa et al. does not disclose the details of the XTT formazan assay, but instead references another article where the details are contained. In this instance, Itokawa et al. reference Weislow et al. J. Natl. Canc. Inst. 81:577-586 (1989), a copy of which is attached hereto.

The XTT assay method is detailed on pages 579 and 580 of Weislow et al. The assay measures the formation of XTT formazan by infected cells or control cells. Tetrazolium salts (e.g. MTT, XTT, WST-1) are used in cell proliferation/viability assays. The tetrazolium salts are cleaved to formazan by the "succinate-tetrazolium reductase" system (EC 1.3.99.1) which belongs to the respiratory chain of the mitochondria and is active only in metabolically active cells. Thus, the Examiner's interpretation of Itokawa et al., i.e. that the reference teaches the direct inactivation of HIV using cell-free viruses in an XTT assay system, is scientifically impossible.

The Examiner bases her interpretation of Itokawa et al. on the statement in the Abstract that,

> The assay procedure is applicable to the evaluation of drug effects on in vitro infections induced either directly in cultured host cells by cell-free HTV-1 or by coculture with H9 cells chronically infected with HIV-1.

However, the reference in this statement in the Itokawa et al. to cell-free HIV indicates that the cells assayed in the XTT formazan assay were infected using either cell-free HIV or H9-infected cells. This statement is not an indicative that the direct killing of cell-free HIV was assayed in Itokawa et al. In addition, Weislow et al. state on page 579, right column,

> Uninfected cells or cells that are protected by drugs and have continued to proliferate produce the soluble orange XTT formazan, (fig. 3A) ... Cells not protected by drugs are killed by the virus...

Thus, the XTT formazan assay is used as an indication of the effectiveness of a drug at protecting a cell from a virus and is not an indication of direct virus killing. As indicated above, it is not scientifically possible to measure the direct killing of a virus using the XTT formazan assay because the assay based on mitochondria metabolic activity.

Thus, in my opinion the bioassay of Itokawa et al. describes the moderate suppression of HIV-1 cytopathic effects on cells, and Itokawa et al. demonstrates that the drugs being tested act on *virus replication* in cell culture. Itokawa et al. does not disclose that the drugs are acting as virus inactivators or that the drugs destroy the infectivity of products by killing the viruses, as achieved with the present invention.

c) Viral infectivity versus viral inactivation: The Examiner interprets the in vitro assay in Naruse et al. that measures viral infectivity as being equivalent to measuring virus

inactivation. The Examiner similarly interprets both Itokawa et al. and Naruse et al. as measuring viral "inactivation", since inhibition of replication would be considered "inactivation". It is my opinion that the Examiner is incorrect in her interpretation of "viral infectivity", "inhibition of viral replication" and "viral inactivation" as meaning the same thing. ["Viral infectivity" is determined in a viability test, that means the ability of a treated virus to infect cells in tissue culture (or animals) is tested. "Inactivation of viruses" means the treatment of viruses by a variety of physical and chemical means (see for both terms: HIV-1 and other Blood-borne Pathogens, in: Diane O. Fleming and Debra L. Hunt (eds.), Biological Safety: Principles and Practices. Washington: ASM Press, 2000, p. 165). The "inhibition of viral replication" is due to the effect of antiviral agents which interfere with the function of viral proteins like AZT inhibits the reverse transcriptase of HIV or the protease inhibitors which inhibit the activity of viral protease and thus prevent the virus maturation, Fundamental Virology, 3rd edition, Eds. Bernard N. Fields, David M. Knipe, Peter M. Howley, Philadelphia/New York: Lippincott-Raven, chapter 26 p. 763 ff.

]

d) <u>Mechanism of drug action in Naruse et al. and the present invention:</u> The Examiner asserts that no evidence has been provided that demonstrates that the mechanism of action with the present invention is different than that of Naruse et al. However, the difference in the mechanisms of drug action with the present invention of Naruse et al. is evident from the experiments of Naruse et al. those of the specification.

Naruse et al. demonstrate that pumilacidins are inhibitors of herpes simplex type 1. See Abstract. In Naruse et al., antiviral activity was assessed using plaque reduction and dye uptake assays with HSV-1-Vero cells. See pages 274-275, *„Antiviral Activity"*. In the experiments of Naruse et al. acyclovir was used as a reference compound for antiviral activity. See page 275, lines 5-6. Acyclovir is well-known in the field of virology to be an inhibitor of viral replication. [Review: Villarreal EC (2001): Current and potential therapies for the treatment of herpesvirus infections. Progress in drug research. Fortschritte der Arzneimittelforschung. Progres des recherches pharmaceutiques; Spec No; 185-228.]

Use of acyclovir as the reference compound in the antiviral experiments evidences that the mechanism of drug action in Naruse et al. is one of inhibition of viral replication, not the direct killing of virus particles, as achieved with the present method.

The mechanism of drug action with the present invention of direct viral killing, is evidenced by at least the experiments of Examples 5, 11 and 12 in the specification. In these experiments, the compounds are mixed with cell-free virus particles and the viruses are killed.

As one skilled in the art, I would also conclude from the total discussions in Naruse et al. that cyclic lipopeptides are not suitable for the inactivation of viruses in protein-containing biological products.

Naruse et al. discuss in the Abstract, on page 275, Table 5 and *"H^+ and K^+ - ATPase Inhibitory Activity"* that pumilacidins A and B are inhibitors of the enzyme acticity of H^+ and K^+ - ATPase. One skilled in the art would predict from this discussion in Naruse et al. that cyclic lipopeptides could not be used for the inactivation of viruses in protein-containing biological products without negatively affecting the biological activity of the products.

In addition, Naruse et al. demonstrate in Table 4, that pumilacidin A and B have ID_{50}'s (concentration for 50% inhibition of cytopathic effects of the non-treated control) that are very similar to the TD_{50}'s, (toxic dose) i.e. a low therapeutic index. This means that the concentrations of pumilacidins required by Naruse et al. for achieving antiviral effects are very close to the toxic concentration. Such a low therapeutic index (difference between the ID_{50} and TD_{50}) would lead one skilled in the art away from using the compounds as antiviral compounds.

I each hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By: /Jung Park/ Date 31 May 2002

Curriculum Vitae of Georg Siegfried Pauli

Studied biology at the University in Cologne with a specialisation in genetics, zoology and biochemistry. In 1972 received the title of Dr. rer. nat. at the Faculty of Natural Sciences of the University at Cologne. The focus of the thesis was on bacterial genetics. Then changed the field of research and joined the research group of Prof. Heinz Bauer focussing on the role of RNA-viruses in the development of cancer.

In 1983 completed habilitation in the field of virology at the Freie Universität in Berlin, Faculty of Veterinary Medicine.

In 1985 awarded a tenure professorship in the Faculty of Veterinary Medicine at The Freie Universität Berlin.

In 1988 became Head of the Laboratory of Virus Diagnostics at the AIDS-Zentrum of the Bundesgesundheitsamt (German Federal Health Office) in Berlin.

In 1994 became Head of the Department of Virology at the Robert Koch-Institut, Berlin.

In 1998 became Head of the Department of Research Coordination and Speaker of the Project Groups "Biology and Epidemiology of Infections". One major focus of research has been the virus safety of blood and blood components and of transplants (s. publications below).

Member of the German Advisory Committee Blood (Arbeitkreis Blut) and the Subgroup "Assessment of Pathogens Transmissible by Blood Infections" (Bewertung von blut-assoziierten Krankheitserregern), Member of the Commission of "Virus Safety" of the German Society of Virology (Gesellschaft für Virologie), and Member of the Commission "Virus Diagnostics" of the German Association against Virus Diseases (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e.V.).

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